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# Inheritance of the Color Patterns of the Blue Snakeskin and Red Snakeskin Varieties of the Guppy, *Poecilia reticulata*

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**ABSTRACT**—The Blue Snakeskin (BSS) and Red Snakeskin (RSS) varieties are popular strains commercially cultured in Singapore. The blue-black tail color of BSS guppies is determined by a dominant X-linked gene (*Blt*) and the silvery snakeskin pattern on the body of males is under the control of a Y-linked gene (*Ssb*). The Y-linked snakeskin tail pattern gene (*Sst*) though present in BSS males is masked by the blue-black tail color gene (*Blt*). The putative genotypes for males and females of the BSS variety are $X_{Blt}Y_{Ssb,Sst}$ and $X_{Blt}X_{Blt}$, respectively. The red tail color of the RSS variety is due to an X-linked dominant gene (*Rdt*). The snakeskin body pattern of RSS males is under the control of the Y-linked *Ssb* gene while the black reticulations on the tail is due to interaction between the snakeskin tail pattern gene (*Sst*) and the red tail gene (*Rdt*). The proposed genotypes for males and females of the RSS variety are $X_{Rdt}Y_{Ssb,Sst}$ and $X_{Rdt}X_{Rdt}$, respectively. An estimate of 0.9% crossover frequency was obtained between the Y-linked *Ssb* and *Sst* genes and a 2.7 % crossover rate of the *Blt* gene from the X- to the Y-chromosome.

## INTRODUCTION

The guppy, *Poecilia reticulata* being a voracious omnivorous feeder and tolerant of polluted waters was introduced to Singapore and other parts Asia for mosquito control [1]. It has been commercially cultured in Singapore since the 1950's and is economically the most important species of freshwater ornamental fish produced almost exclusively for the export market. The wide variation of brilliant and beautiful colors on the body, tail and dorsal fin of male guppies makes it one of the most popular and ubiquitous ornamental fish. About 30 domesticated varieties of guppies are produced on monoculture farms in Singapore. Each farm specialises in 8–12 varieties [2]. Guppy farmers continuously strive to improve the quality of the fish and to breed new varieties with novel color patterns. To do so it is important to understand the gene control of the color phenotypes of domesticated guppies. So far there are few reports on the inheritance of color patterns of domesticated varieties of the guppy [3–7]. The objective of this study is to elucidate the gene control of the color phenotype of the Blue Snakeskin and Red Snakeskin varieties of *P. reticulata* cultured in Singapore.

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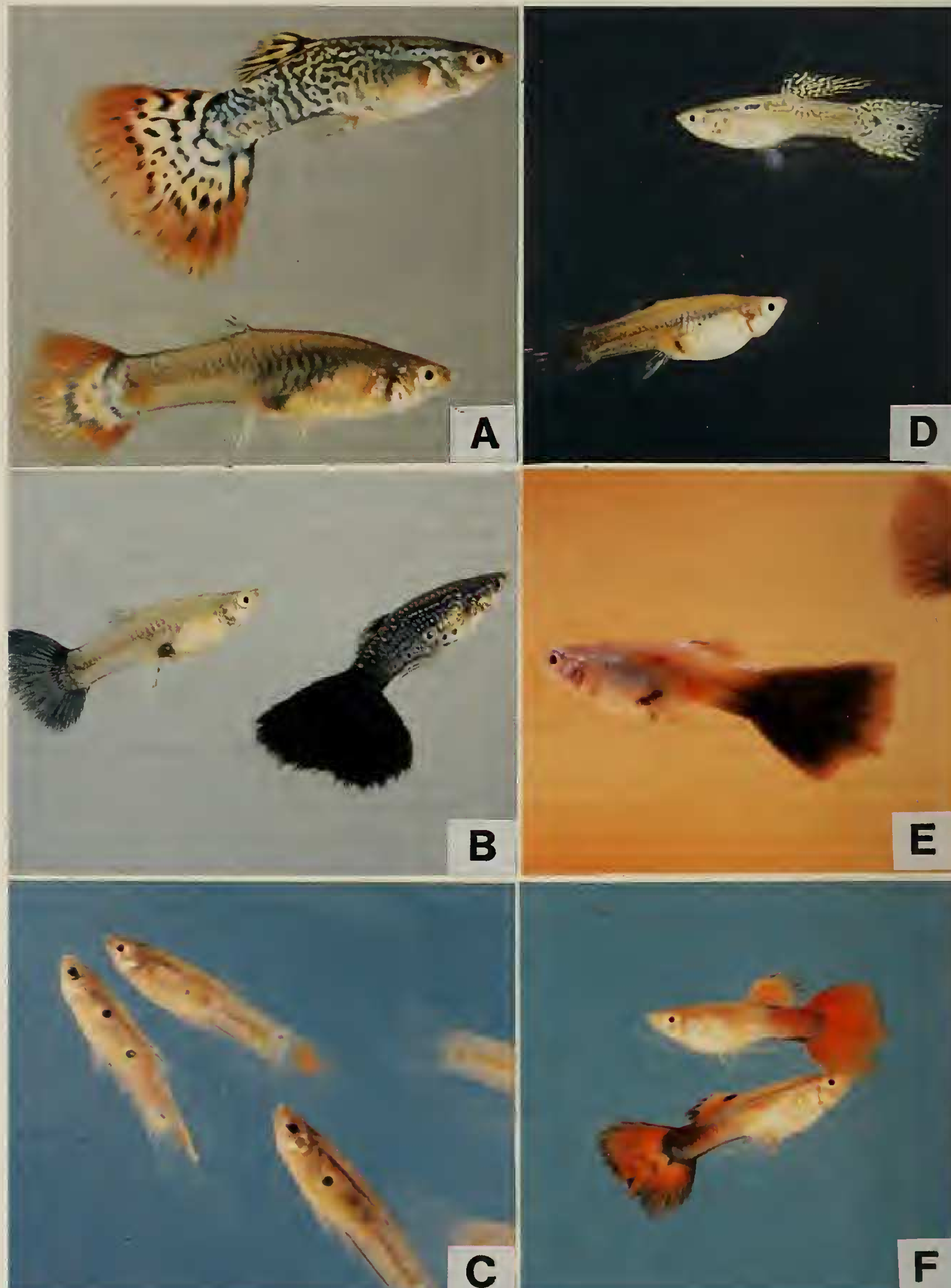
## MATERIALS AND METHODS

### *Source of the fish*

Two- to three-weeks old fry of the Blue Snakeskin (BSS) and Red Snakeskin (RSS) varieties were obtained from the Lim Chin Lam Guppy Farm in Singapore. Wild-type (WT) fry were collected from a stream in a rural area of Singapore. Since virgin females were required for the reciprocal crosses, fry were raised in 33 liter clear plastic tanks (20 fish/tank) in the aquarium area of the Department of Zoology, National University of Singapore, at temperatures of 26–28°C. Sexual differentiation takes place at 4–6 weeks of age under laboratory conditions. The young fish were checked daily for developing


A
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males which are recognized by the modification of the anal fin into the gonopodium. Males when spotted were immediately removed and raised separately from females.

*Description of the varieties*

Adult males and females of the BSS and RSS varieties have total length of 4–5 cm. Adult RSS males have iridescent snakeskin-like reticulations on the olive-brown body and an orange-red colored tail with black reticulations (Fig. 1A). BSS males are also characterized by silvery snakeskin-like pattern on the body but the tails are blue-black in color (Fig. 1B). Both RSS and BSS females lack the snakeskin patterns. RSS females have the normal wild-type olive brown body coloration with tinges of red and opaque white on the tail. BSS females have the WT body coloration and partial expression of the blue-black tail color.

Wild-type guppies are smaller than the domesticated varieties, with marked size differences between males and females. Adult WT females are 3.0–3.5 cm long while adult males are about 2 cm long. WT males have highly polymorphic color patterns, consisting of spots or patches of various colors on the body, tail and sometimes the dorsal fin while the females lack color patterns (Fig. 1C).

*Reciprocal crosses*

The inheritance of the color phenotypes of the BSS and RSS varieties were elucidated by performing single-pair reciprocal crosses between each of them and the wild-type stock, using 3-month old sexually mature virgin fish. The pairs were kept in eight liter breeding tanks. The following notations were used for the crosses:

Cross 1A: BSS male×WT female, Cross 1B: WT male×BSS female

Cross 2A: RSS male×WT female, Cross 2B: WT male×RSS female

Broods were usually produced 4–6 weeks after mating. $F_2$ broods were obtained from single-pair matings between full-sib $F_1$ fish. Phenotypic proportions among the $F_1$ and $F_2$ progeny were subjected to chi-square tests.

Twenty adult females of the BSS and RSS parental stocks and all the $F_1$ and $F_2$ female progeny that were not required for breeding were fed with the androgen, methyl testosterone, to express any inherent color genes [8, 9].

## RESULTS

*Androgen treatment of BSS and RSS females*

When androgen treated the tail color of BSS females deepened to a dark blue no snakeskin pattern was manifested on the body. Androgen treated RSS females developed red color on the tail but not the iridescent snakeskin body pattern or the black reticulations on the tail. These results showed that the BSS and RSS females carried the genes for the blue tail color and red tail color, respectively, but not the genes determining the snakeskin pattern.

*Cross between the BSS variety and WT stock*

Twelve matings between BSS males and WT females (Cross 1A) gave a total of 120 male and 150 female $F_1$ progeny (Table 1). The $F_1$ males had the hyaline tail color of WT guppies and a delicate, silvery snakeskin pattern on the body and tail (Fig. 1D). We called the color phenotype of these $F_1$ males wild-type snakeskin (WTSS). The $F_1$ females had blue tinges on the tail which on androgen treatment deepened in color but no snakeskin pattern developed the body or tail. These females were cinsidered to have the blue tail phenotype (BT). Since only the male progeny inherited the snakeskin pattern from the BSS male

FIG. 1.A. Adult male (upper) and female (lower) of the Red Snakeskin variety of the guppy.
FIG. 1.B. Adult female (left) and male (right) of the Blue Snakeskin variety of the guppy.
FIG. 1.C. Three adult male wild-type guppies.
FIG. 1.D. A $F_1$ male of the cross between BSS males and WT females showing delicate iridescent snakeskin pattern on the body and tail and hyaline wild-type tail (wild-type snakeskin phenotype) (upper). A $F_1$ female of this cross (lower).
FIG. 1.E. A $F_1$ male of the cross between WT males and BSS females showing blue tail (BT) phenotype.
FIG. 1.F. Two $F_1$ male of the cross between WT males and RSS females showing the red tail (RT) phenotype.

TABLE 1. $F_1$ and $F_2$ segregation data of reciptrocal crosses between the Blue Snakeskin variety and the wild type stock

| Cross (Gross No.) | Gen. | No. matings (No. broods) | No. & Phenotypes of Progeny Males | Females | Exp. ratio | $\chi^2$ |
|---|---|---|---|---|---|---|
| BSS♂×WT♀ (Cross 1A) | $F_1$ | 12 (12) | 120 WTSS | 150 BT | 1:1 | 3.33 |
| | $F_2$ | 10 (15) | 46 WISS | 51 BT | 1:1:1:1 | 1.92 |
| | | | 60 BSS | 53 WT | | |
| | | | §2 WISST | | | |
| WT♂×BSS♀ (Cross 1B) | $F_1$ | 12 (12) | 97 BT | 108 BT | 1:1 | 0.59 |
| | $F_2$ | 12 (15) | 76 BT | 178 BT | *1:1:2 | 2.86 |
| | | | 72 WT | #5 WT | | |

§ Exceptional $F_1$ males with wild type body and snakeskin tail pattern (*Sst*).
\# Exceptional $F_2$ females (Cross 1B) with hyaline tail color.
* Exp. ratio for the typical $F_2$ offspring of Cross 1B.

parent, it showed Y-linkage of the genes determining the snakeskin pattern. The presence of snakeskin reticulations on the tail of $F_1$ males showed that the BSS male parents were carrying the gene for snakeskin tail pattern which was masked by the blue-black tail color. The snakeskin body and tail patterns of another guppy variety, the Green Snakeskin, are controlled by two closely linked genes (*Ssb* and *Sst*) on the Y-chromosome [10]. Absence of the blue tail color of the BSS male parents in $F_1$ males and presence in all $F_1$ females gave evidence of X-linkage and dominance of the gene determining the blue tail coloration which has been designated as *Blt* [11]. The recessive allele, $Blt^+$, present in WT guppies gives the hyaline tail color.

The $F_2$ generation of Cross 1A cinsisted of BSS males, WTSS males, BT females and WT females with observed numbers conforming to the 1:1:1:1 expected ratio (Table 1). These results gave evidence that the putative color genotype of BSS males is $X_{Blt}Y_{Ssb,Sst}$ and that for WTSS males is $X_{Blt}+Y_{Ssb,Sst}$. A genetic model is proposed to show segregation of the color genes in Cross 1A (Fig. 2).

However, there were two exceptional $F_2$ males with WT body color and snakeskin pattern on the tail giving further evidence that the snakeskin body and tail patterns are determined by two Y-linked genes, *Ssb* and *Sst,* respectively. The absence of the expected snakeskin body pattern in these two males is probably due to crossing-over of the *Ssb* gene irom the Y- to the X-chromosome. The crossover frequency between the *Ssb* and *Sst* genes calculated from the $F_2$ offspring of Cross 1A is 0.9%, two crossovers out of a total of 212 $F_2$ individuals.

Twelve matings of the reciprocal cross (Cross 1B) between WT males and BSS females gave 12 $F_1$ broods consisting of 97 males and 108 females, all with blue tails (BT phenotype) and without any snakeskin pattern (Fig. 1E). With the exception of five females with the WT phenotype, the $F_2$ progeny of this cross segregated into BT males, WT males and BT females according to the 1:1:2 hypothetical ratio. Thus the $F_1$ and $F_2$ results gave evidence that the BSS parental females were homozygous for the X-linked dominant blue tail gene (*Blt*) with the genotype being $X_{Blt}X_{Blt}$. Figure 2 shows the proposed genetic model for the segregation of color genes in Cross 1B.

The occurrence of five exceptional $F_2$ females of Cross 1B, with hyaline tails instead of the expected blue tail color of the typical $F_2$ females could be due to crossing over of the X-linked *Blt* gene to the Y-chromosome in the $F_1$ male parents of these individuals. Since there were five crossover females out of a total of 183 $F_2$ females of Cross 1B, the crossover frequency of the *Blt* gene from the X- to the Y-chromosome in the $F_1$ females of this cross is 2.7%. Crossovers among the $F_2$ males cannot be detected (Fig. 2).

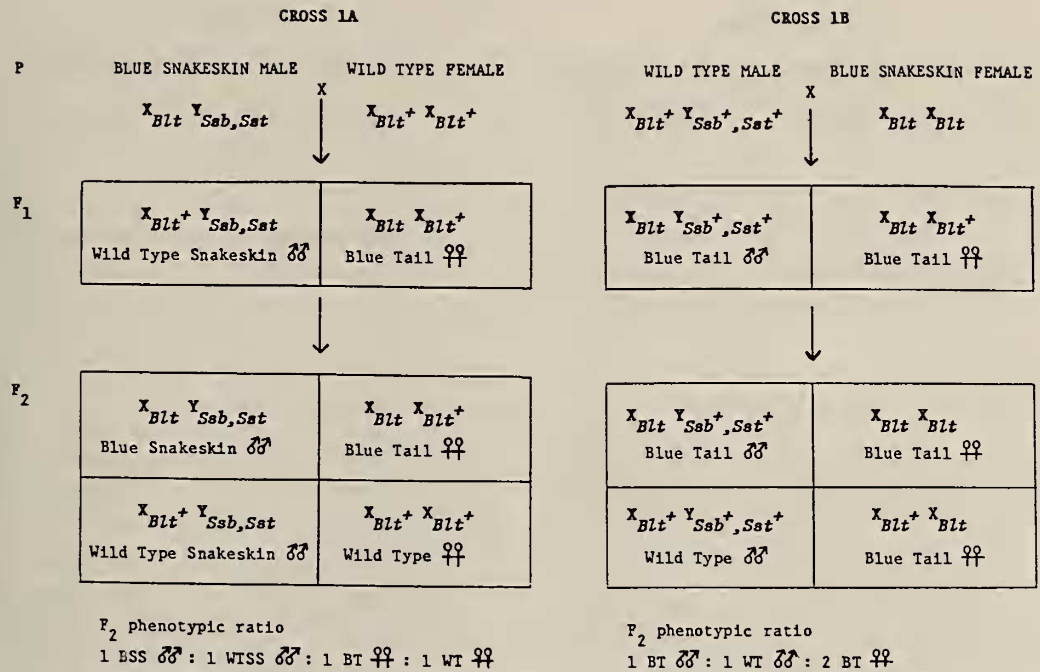


FIG. 2. Schematic diagram of the proposed genetic model for the segregation of color genes in the $F_1$ and $F_2$ generations of reciprocal crosses between the Blue Snakeskin variety and wild-type guppies.

TABLE 2. $F_1$ and $F_2$ segregation data of reciprocal crosses between the Red Snakeskin variety and the wild type stock

| Cross (Gross No.) | Gen. | No. matings (No. broods) | No. & Phenotypes of Progeny: Males | Females | Exp. ratio | $\chi^2$ |
|---|---|---|---|---|---|---|
| BSS♂×WT♀ (Cross 2A) | $F_1$ | 6 (12) | 117 WTSS | 135 BT | 1:1 | 1.28 |
| | $F_2$ | 5 (13) | 74 RSS | 98 RT | 1:1:1:1 | 4.39 |
| | | | 76 WISS | 80 WT | | |
| WT♂×BSS♀ | $F_1$ | 5 (10) | 94 RT | 122 RT | 1:1 | 3.63 |
| (Cross 2B) | $F_2$ | 6 (12) | 56 RT | 140 RT | 1:2:1 | 2.21 |
| | | | 70 WT | | | |
| *WT♂×RSS♀ (Cross 2B) | $F_1$ | 1 (3) | 16 RT | 22 RT | 1:1:1:1 | 4.06 |
| | | | 10 WTSS | 12 WTSS | | |

* Exceptional mating between a RSS♀ producing three exceptional broods, giving evidence that the RSS female parent was heterozygous for the *Ssb* and *Sst* genes.

*Cross between the RSS variety and WT stock*

Six matings between RSS males and WT females (Cross 2A) produced 12 $F_1$ broods (Table 2). The 117 $F_1$ males had hyaline tails and iridescent snakeskin pattern on the body and tail (WTSS phenotype) like the $F_1$ males of Cross 1A. The 135 $F_1$ females showed pink tinges on the tail which deepened to red after androgen treatment (RT phenotype). The pooled $F_1$ data conformed to the 1 WTSS male: 1 RT female. Thus, results showed that the RSS parental males carried the dominant X-linked red tail gene (designated as *Rdt*) which they passed to their daughters and the Y-linked snakeskin body (*Ssb*) and snakeskin tail (*Sst*) genes which were transmitted to their sons. The recessive tail color allele, $Rdt^+$ gives the hyaline tail color. The $F_2$ progeny of this cross segregated into

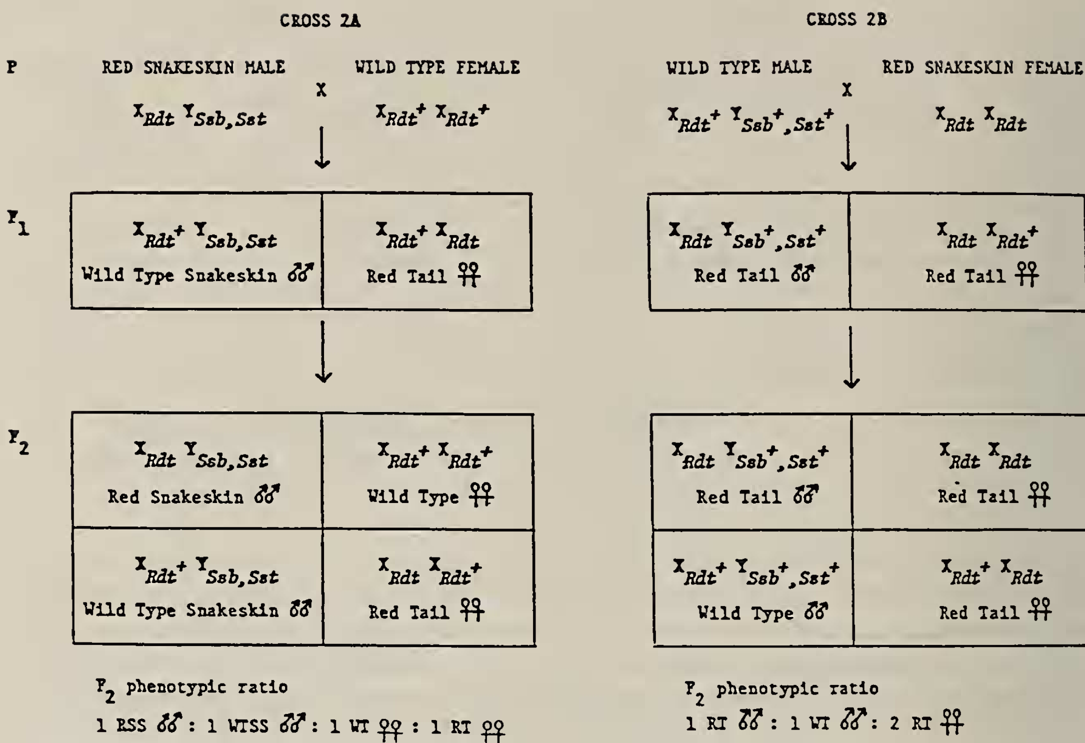


FIG. 3. Schematic diagram of the proposed genetic model for the segregation of color genes in the $F_1$ and $F_2$ generations of reciprocal crosses between the Red Snakeskin variety and wild-type guppies.

four phenotypic classes: red snakeskin males (RSS), wild-type snakeskin males (WTSS), red tail females (RT) and WT females with observed numbers conforming to the expected 1:1:1:1 ratio. The putative genotype of RSS males is $X_{Rdt}Y_{Ssb,Sst}$ and that for WTSS males is $X_{Rdt^+}Y_{Ssb,Sst}$. A genetic model is proposed to show the segregation of color genes in Cross 2A (Fig. 3).

Five matings of the reciprocal cross of WT males with RSS females (Cross 2B) gave 10 typical $F_1$ broods consisting of red tail (RT) males and females with numbers conforming to the expected 1:1 segregation ratio (Fig. 1F). The phenotypes of the females were expressed after androgen treatment. The 12 $F_2$ broods consisted of 56 RT, 70 WT males and 140 RT females. These observed numbers fit an expected segregation ratio of 1:1:2 and provided evidence that the RSS parental females were homozygous for the X-linked dominant red tail gene, *Rdt* ($X_{Rdt}X_{Rdt}$ genotype).

A single atypical mating of Cross 2B gave three exceptional $F_1$ broods consisting of four phenotypic classes: expected RT males, unexpected WTSS males, expected RT females and unexpected WTSS females. The observed numbers segregated according to the hypothetical 1 : 1 : 1 : 1 ratio (Table 2). These results give evidence that the exceptional RSS female parent of this mating carried the *Ssb* and *Sst* genes on one of the X-chromosomes.

## DISCUSSION

The blue-black tail color of the BSS variety is found to be controlled by the X-linked *Blt* gene and the red tail color of RSS guppies by the X-linked *Rdt* gene. There is similar evidence of X-linkage of the blue tail and red tail color tail genes present in the domesticated Blue Tail variety and Red Tail variety, respectively [6]. Fernando *et* *al.* [11] also reported Y-linkage of these genes in domesticated varieties from one farm in Singapore. In this study 2.7% of crossing over of the *Blt* gene from the X- to the Y-chromosome was found, while no crossovers were observed for the *Rdt* gene. Crossing over of genes from the X- to the Y-chromosome and *vice versa* in the guppy was first documented by Winge [12] and the crossover data have been used to map the sex chromosomes [3, 5, 13, 14]

In male guppies with wild-type hyaline tails (*Blt*+and *Rdt*+) the snakeskin tail pattern gene (*Sst*) is expressed as iridescent delicate reticulations. The *Sst* gene in males carrying the red tail gene (*Rdt*) is manifested as black reticulations on the orange-red tail. However, in males carrying the *Blt* gene which gives the blue-black tail color, the *Sst* gene when present is completely masked. Outcrosses with WT females showed that the BSS male guppies are carrying the *Sst* gene.

The 0.9% recombination frequency between the Y-linked *Ssb* and *Sst* genes found among the $F_2$ progeny of Cross 1A is in close agreement with the 1% reported in another domesticated guppy variety, the Green Snakeskin [10]. No crossovers were found among the progeny of Cross 1A showing that the *Ssb* and *Sst* genes tend to behave as a supergene and are transmitted as a single unit on the Y-chromosome through the male line. The single RSS female parent of Cross 2B heterozygous for the *Ssb* and *Sst* genes, gave evidence that one or both these normally Y-linked genes could be found on the X-chromosome due to crossing-over. Our results also showed that the *Blt, Ssb* and *Sst* genes are found on the homologous segments of the sex chromosomes. Currently experiments are being conducted to test for possible allelism of the blue tail (*Blt*) and red tail (*Rdt*) color genes.

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## REFERENCES

1 Herre, A. W. C. T. (1940) Additions to the fish fauna of Malaya and notes on rare and little known Malayan and Bornean fishes. Bull. Raffles Mus. Sing., **16**: 27–61.

2 Fernando, A. A. and Phang, V. P. E. (1985) Culture of the guppy, *Poecilia reticulata* in Singapore. Aquaculture, **51**: 49–63.

3 Dzwillo, M. (1959) Genetische Untersuchungen an domestizierten Stammen von *Lebistes reticulatus* (Peters). Mitt. Hamburg Zool. Mus. Inst., **57**: 143–186.

4 Dzwillo, M. (1962) Uber kunstliche erzeung funktioneller Mannchen weiblichen Genotyp bei *Lebistes reticulatus.* Biol. Zentrabl., **81**: 575–584.

5 Nayudu, P. L. (1979) Genetic studies of melanic color patterns and atypical sex determination in the guppy, *Poecilia reticulata.* Copeia, **2**: 225–231.

6 Phang, V. P. E., Chow, O. K. and Fernando, A. A. (1985) Genetic analysis of scale chromatophores of two domesticated varieties of the guppy, *Poecilia reticulata.* J. Sing. Nat. Acad. Sci., **14**: 1–5.

7 Phang, V. P. E., Fernando, A. A. and Chow, O. K. (1986) Inheritance of body and tail coloration in two domesticated varieties of the guppy. In "Genetics in Aquaculture II". Ed. by G. A. E. Gall and C. A. Busack, Elsevier, Amsterdam, p. 372.

8 Hildemann, W. (1954) The effects of sex hormones on secondary sex characters of *Labistes reticulatus.* J. Exp. Zool., **121**: 1–5.

9 Fernando, A. A. and Phang, V. P. E. (1985) Expression of sex-limited color pattern genes in selected strains of female guppies *Poecilia reticulata* with androgen treatment. Sing. Nat. Acad. Sci., **14**: 157–159.

10 Phang, V. P. E., Ng, L. N. and Fernando, A. A. (1989) Inheritance of the snakeskin pattern in the guppy. J. Hered. (In press)

11 Fernando, A. A. and Phang, V. P. E. (1988) Evidence for X- and Y-linkage of a tail colour gene in a domesticated variety of the. guppy, *Poecilia reticulata.* J. Sing. Nat. Acad. Sci. (In press)

12 Winge, O. (1923) Crossing over between the X- and Y-chromosome in *Lebistes.* C. R. T. Lab. Carlsberg, **14**: 1–19.

13 Winge, O. (1934) The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by *Lebistes.* C. R. T. Lab. Carlsberg, **21**: 1–49.

14 Winge, O. and Ditlevsen. E. (1947) Color inheritance and sex determination in *Lebistes. Heredity* **1**: 65–83.